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Strengthening Drought Resistance in Grass Peas via Plant-Smoke Solutions

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Abstract: In this study, the negative effect of drought severity on forage quality in grass pea (*Lathyrus sativus* L.) genotypes and the role of smoke solution in preventing this effect were investigated. For this purpose, seeds primed with two different concentrations of poppy smoke solution were grown in three different environments and pots, including normal irrigation, moderately severe, and severe drought for 28 days. The trials were conducted under controlled conditions in the climate chamber separately for each genotype and environment. After harvest, shoot length, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and mineral contents (calcium, magnesium, phosphorus and potassium) ratios were determined. As a result, it was observed that the protein content increased under drought stress for both genotypes, but this increase had a negative effect on mineral content, ADF, and NDF. The application of smoke solution prevented this negative effect and even further increased CP. The highest CP rate was determined as 35.48% at 10% solution dose in moderate drought and 35.12% at the same dose in severe drought. Furthermore, positive effects of smoke solutions at both doses on quality were determined under normal irrigation conditions. Additionally, the population's resistance to drought and response to smoke solutions were higher than the variety. In conclusion, it was revealed that applying a 10% smoke solution in grass pea genotypes has a preventive effect on drought stress.

Keywords: Smoke solution, priming, crude protein, drought, mineral content

1. Introduction

Drought stress refers to the adverse effects on plants caused by prolonged or severe water scarcity. It occurs when there is an insufficient supply of water to meet the needs of plants for their normal growth and development. In the future, drought will become an increasingly critical concern, especially in light of ongoing climate change and its expected consequences. Frankly, drought, among other abiotic stresses, stands out as a meteorological event exhibiting a rising trend in terms of duration, frequency, and spatial impact, significantly affecting agricultural production.

Drought significantly hampers the morph-physiological functions of plants, negatively impacting growth, productivity, reproduction, and survival (Zandalinas et al., 2020). Plants exhibit adaptability or responses to changing environmental conditions through diverse morphological, physiological, anatomical, and biochemical changes. Plants exhibit varying degrees of tolerance to drought stress, and different species or varieties may have specific adaptations to cope with water scarcity. Understanding the mechanisms of drought stress and developing strategies to enhance plant resilience are crucial for mitigating the impact of water scarcity on agriculture and natural ecosystems. Developing drought-resistant traits in existing plant genotypes is essential for reducing the impact of drought on agricultural practices. However, mechanisms and responses specific to drought stress can vary widely among genotypes. The grass pea (*Lathyrus sativus* L.) stands out as a rich source of protein, iron, potassium (K) and calcium (Ca) in both grain and hay form. In addition, grass peas are particularly resistant to drought stress, but not in severe drought conditions. Strengthening the mechanism developed by grass pea in dry conditions with smoke solutions can also increase its use in severely dry areas. Therefore, this study focused on examining two different genotypes of grass pea under drought conditions (Seleiman et al., 2021).

To convert arid lands into fertile agricultural areas, it is essential to prioritize sustainable farming practices. Various techniques have been employed to alleviate drought stress, including film farming, the development of drought-resistant crops (Nuccio et al., 2018), the use of nanoparticles (Saxena et al., 2016), and the application of superabsorbents, hydrogels, and biochar (Saha et al., 2020; Zhang et al., 2020). Additionally, the use of plant growth-promoting rhizobacteria has shown considerable potential (Chiappero et al., 2019). Among these strategies, plant-derived smoke solutions stand out as a relatively novel and underexplored approach within agricultural stress mitigation. These solutions involve repurposing agricultural waste to combat drought stress, thereby contributing to environmental sustainability and global food security. Frankly, smoke solution applications, known for effects on germination and plant development, play a crucial role in mitigating drought stress in plants by enhancing their resilience and adaptation mechanisms (Dixon et al., 2009). These solutions contain compounds that trigger specific physiological responses in plants, such as improved water uptake efficiency, increased antioxidant activity, and altered hormone levels, which collectively help plants cope with water scarcity (Bose et al., 2020). Smoke solutions have been discovered to enhance plant resilience against a range of abiotic stresses, such as drought (Li et al., 2017), flooding, cold temperatures (Shah et al., 2021), cadmium toxicity (Shah et al., 2020), and salinity (Khan et al., 2017). These solutions, derived from plant materials, play a significant role in fortifying plants' ability to withstand challenging environmental conditions. By improving their tolerance to these stressors, smoke solutions contribute to the overall health and productivity of crops, making them a valuable tool in the quest for sustainable agricultural practices. In this study, the ameliorative/inhibitory effect of smoke solution on hay quality in normal cultivation and under different drought intensities in grass pea (*L. sativus*) genotypes was investigated.

2. Materials and Methods

The study utilized two grass pea (*L. sativus*) genotypes: one originating from a population in Türkiye and the other from a registered variety known as "Karadağ". As part of an environmentally conscious approach, repurposed poppy harvest residues to create a smoke solution. This innovative method demonstrates a sustainable use of agricultural by-products, highlighting the importance of recycling in modern agricultural practices. The experimentation in controlled climate room within the Agriculture Faculty of Yozgat Bozok University, where environmental conditions such as light, temperature, and humidity were fully regulated.

Smoke solutions were created from poppy residues by burning 1 kg of poppy straw in a controlled manner using a specialized system, following the methods outlined in studies by Ghebrehiwot et al. (2009) and Başaran et al. (2019). The resulting smoke was captured by passing it through 4 liters of distilled water. This initial smoke solution was then diluted to concentrations of 1% and 10% using additional distilled water. Grass pea seeds were soaked in these smoke solutions for 18 hours at a temperature of 22 °C, with distilled water used as the control. The primed seeds were placed in the growing medium without waiting. The 8 L pots filled with equal amounts of peat and soil (1:1) were used as the growing medium.

The study included three treatment groups; normal irrigation (control, C), moderate drought (M), and severe drought (S). Severe drought stress was imposed by withholding water for the last 8 days, while moderate drought stress involved withholding water for the last 4 days. The primed seeds of plants were planted in pots. That is; 2 separate trials were set up for 2 different genotypes with 2 different priming applications and 3 different drought levels. Using random plots factorial experimental design, the experiment included three replications, each with 10 plants in pot. Both grass pea varieties were carefully cultivated under controlled conditions, maintaining a temperature of 25 ± 2 °C. All plants were then harvested 28 days after the start of the experiment.

The 28 days later, all plants were removed from the pots, and their shoot lengths (cm) were measured. The samples were then dried in a cabinet at 60 ºC for 48 hours and ground to a diameter of 1 mm. After grinding, the samples were passed through a 1 mm sieve. Subsequently, the ground samples were analyzed using a near infrared reflectance spectroscopy (NIRS) device (Foss 6500, Silver Spring, MD, USA) with the IC0904FE program to determine crude protein (CP, %), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), and mineral matter content [Ca, phosphorus (P), K, and magnesium (Mg), %].

The data obtained were analyzed using the SPSS 2.0 package program according to the random plots factorial experimental design. Differences between means were grouped using the Duncan multiple comparison test at the p<0.05 level. Additionally, principal component analysis (PCA) analysis was conducted using the XTAT analysis program, and a graph was created. Correlation, heat map, and balloon plot graphs were generated using SRplot (Tang et al., 2023).

3. Results

The changes in plant heights of 28-day-old seedlings grown in different environments are presented in Figure 1. As expected, the shoot lenght decreased in both genotypes with increasing severity of drought conditions. The applied smoke solutions had a negative effect in all three environments, further reducing the shoot lenght. This reduction was parallel to the solution dose (Figure 1).

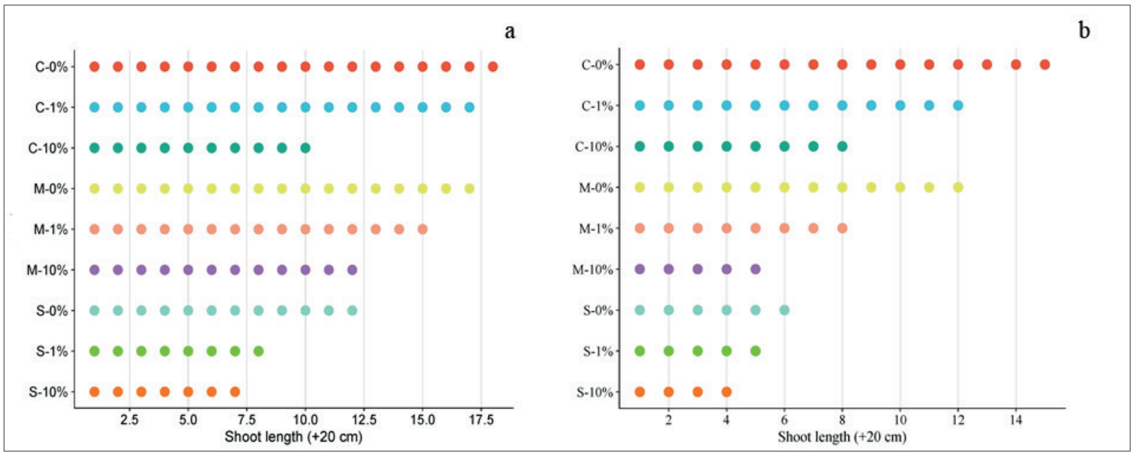

Figure 1. Shoot length change of grass pea genotypes (a: Variety, b: Population) under normal irrigation (C), moderate (M) and severe (S) drought environments

All chemical contents examined in both genotypes were found to be statistically significant (p<0.01) (Table 1 and 2). The CP of the variety was observed to partially increase under severe drought. However, this increase was supported by smoke solution applications. Thus, the highest CP was determined in the 10% solution applications of both drought environments. Furthermore, smoke solution, in both doses, had a positive effect under normal irrigation conditions (control). While the ADF ratio increased under stress conditions, this increase was reduced with smoke solution. The NDF ratio exhibited different responses under drought intensities, but smoke solutions reduced NDF similarly. When Ca, Mg, P, and K minerals were evaluated together, maximum values were observed in the S-10% application. This result clearly demonstrates that smoke solution application improves mineral content, especially under severe drought. Additionally, P and K ratios also achieved maximum values in the 10% solution application of moderate drought (Table 1).

The CP ratio of the population was similar to that of the variety. However, the effect of smoke solution was more pronounced and positive both under drought conditions and under normal conditions (control). The highest value for ADF

Table 1. Chemical compounds (%) of grass pea variety under normal irrigation (C), moderate (M) and severe (S) drought environments*

Treatments	CP	ADF	NDF	Ca	Mg	P	K
C-0%	32.13 c	27.34 b	37.56 ab	1.05 bc	0.20 a	0.53 bc	4.19 cd
C-1%	33.53 b	27.06 b	35.08 bc	1.05 bc	0.16 bc	0.53 bc	4.14 d
C-10%	33.65 b	27.23 b	36.09 b	1.05 bc	0.16 bc	0.52 c	4.27 c
M-0%	32.14 c	28.47 a	38.55 a	1.05 bc	0.15 c	0.52 c	4.25 c
M-1%	32.96 bc	27.66 b	37.77 ab	1.00 c	0.16 bc	0.54 b	4.32 b
M-10%	35.48 a	23.02 c	31.56 c	1.05 bc	0.13 d	0.56 a	4.61 a
S-0%	32.57 bc	28.27 a	35.90 b	1.09 b	0.17 b	0.54 b	4.18 cd
S-1%	32.96 bc	26.58 bc	34.74 bc	1.07 b	0.19 a	0.53 bc	4.29 c
S-10%	35.12 a	21.51 d	26.77 d	1.24 a	0.19 a	0.55 ab	4.46 ab

*: Different letters significant differences at p≤0.05 within one parameter

Table 2. Chemical compounds (%) of grass pea population under normal irrigation (C), moderate (M) and severe (S) drought environments*

Treatments	CP	ADF	NDF	Ca	Mg	P	K
C-0%	27.56 c	24.88 c	42.32 a	1.19 a	0.12 d	0.46 d	3.77 d
C-1%	33.71 b	27.24 b	39.70 a	0.99 d	0.13 cd	0.54 b	4.09 c
C-10%	34.97 a	24.14 cd	34.78 c	1.10 b	0.21 a	0.53 bc	4.28 bc
M-0%	31.09 bc	29.09 a	40.12 a	1.01 c	0.18 b	0.51 c	4.25 bc
M-1%	33.57 b	23.10 cd	32.98 c	1.11 b	0.16 bc	0.55 ab	4.41 b
M-10%	34.97 a	27.43 b	38.52 b	0.98 d	0.16 bc	0.53 bc	4.27 bc
S-0%	32.97 b	25.79 c	34.83 c	1.04 c	0.13 cd	0.55 ab	4.42 b
S-1%	34.98 a	22.13 d	27.97 d	1.14 ab	0.15 bc	0.56 a	4.59 a
S-10%	34.10 a	25.55 c	32.79 c	1.07 bc	0.15 bc	0.56 a	4.42 b

*: Different letters significant differences at p≤0.05 within one parameter

ratio was determined in the M-0% (29.09%), while the lowest value was found in the S-1% treatment (22.13%). For the NDF ratio, the lowest value was identified in the S-1% treatment (27.97%). The population's mineral content responses to stress conditions and smoke solutions were variable. The calcium ratio was obtained from the control 0 dose and the severe drought 1% dose. The highest magnesium ratio was observed in the C-10% (0.21%), while the potassium ratio was determined in the S-1% (4.59%) treatment (Table 2).

In this study, we utilized PCA to delve deeper into the complexity of 8 variables and uncover their fundamental characteristics. Moreover, PCA was employed to simultaneously observe the effects of all transactions on the graph for each genotype, using two components. The PCA biplots offered a comprehensive overview of grass pea's biochemical responses to water deficiency and smoke solutions. We created separate PCA biplots for the population and variety of grass pea, encompassing all the investigated traits. The biplot graphs for variety explained 83.8% of the cumulative variance, with PC1 and PC2 contributing 62.7% and 21.1% of the cumulative variance, respectively. In contrast, the population exhibited principal components PC1 and PC2, which represented 57.6% and 24.4% of the cumulative variance, respectively (Figure 2).

Regarding the vector contributions in the biplot for the genotypes, the first component was heavily loaded by almost all the traits, and the second component too, except for Mg. It can be noted that there are 4 main groups in population biplot and 3 main groups in variety biplot, as indicated groups in the heat map (Figure 3). There were 4 groups formed due to the effect of drought stress and smoke solutions, in both genotypes. However, the relationships differed primarily based on the dose of smoke solutions. Thus, priming, especially with smoke solution, led to a decrease in this basic distinction, and different treatments were separated. Particularly, S-10 and M-10% in variety, S-1 and M-1% in the population were placed in the same group (Figure 3).

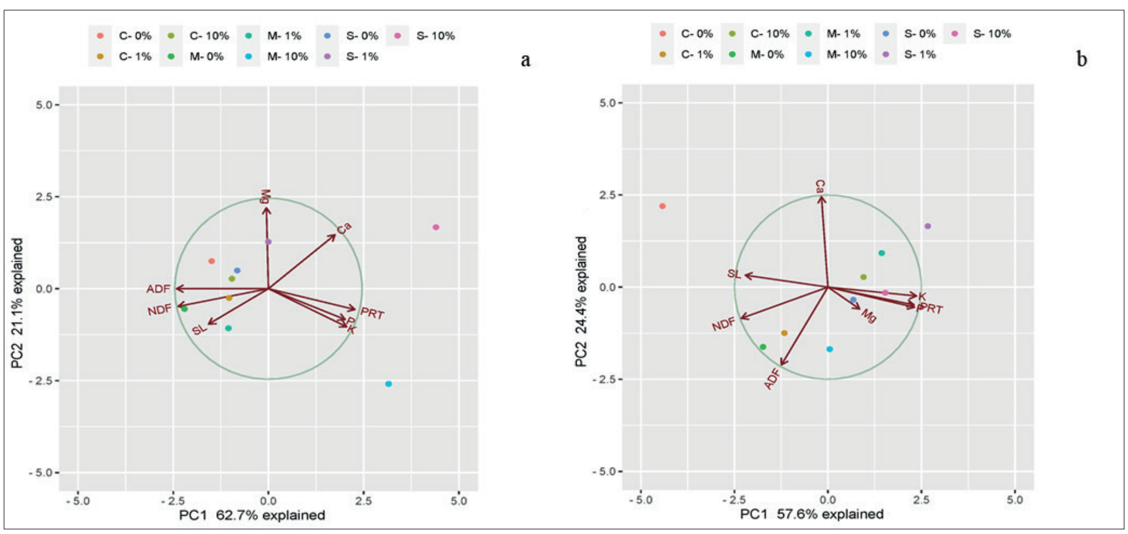

Figure 2. PCA biplots of the responses of smoke solution applied to grass pea under normal irrigation, moderate and severe drought environments (a: Variety, b: Population)*

*: The mean values of the replicates are shown

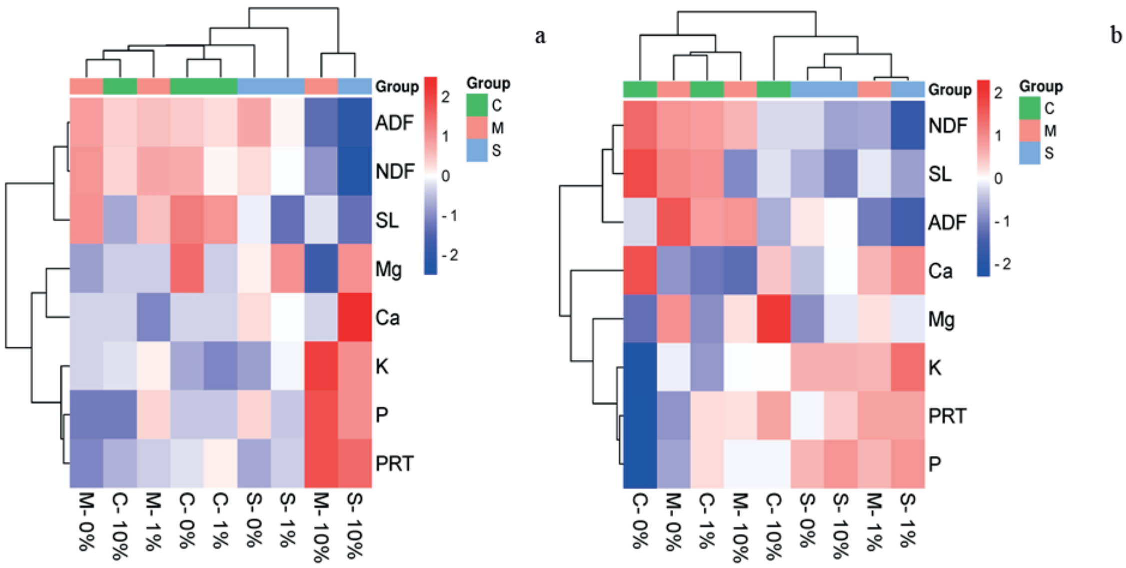

Figure 3. A heat map graph of in grass pea genotypes under normal irrigation, moderate and severe drought environments (a: Variety, b: Population) (Anonymous, 2024)

Furthermore, it was evident that the response mechanism to drought stress in the genotypes associated with an increase in CP, K, and P. However, according to ballon plots, CP, shoot length, ADF and NDF properties show that it is more effective in terms of weight (Figure 4). When figures 2, 3 and 4 are evaluated together, these treatments highlight the remarkable efficacy of the poppy smoke solution in enhancing drought stress tolerance.

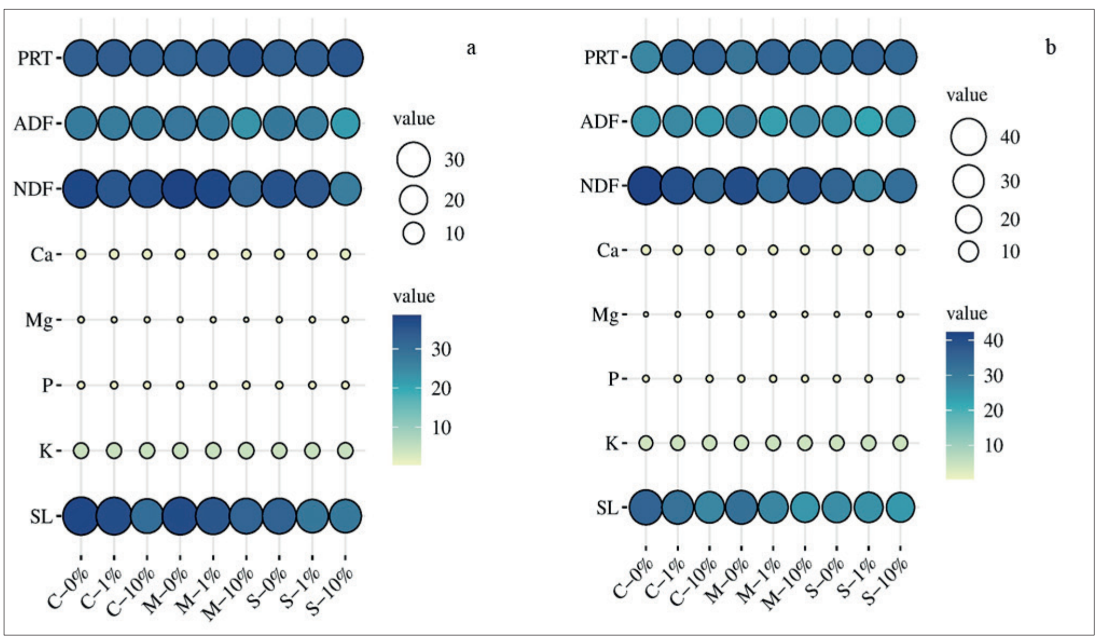

Figure 4. Representation with the balloon plot of the responses of grass pea genotypes under normal irrigation, moderate and severe drought environments (a: Variety, b: Population)*

*: The mean values of the replicates are shown

4. Discussion and Conclusion

The study investigated the effects of drought stress and smoke solutions on the chemical responses of grass pea varieties. The results showed that increasing severity of drought conditions led to a decrease in shoot length both genotypes and smoke solutions further reduced shoot length in all environments. This expected result, increasing severity of drought conditions led to a decrease in shoot length in both genotypes, which aligns with previous studies (Reinhardt et al., 2015). The negative effect of smoke solutions on shoot length, observed in all three environments, is consistent with the findings of (Başaran et al., 2019; Doğrusöz et al., 2022), where smoke solutions were found to decrease shoot length in various plant species.

Chemical contents examined in both genotypes were statistically significant. The CP of the variety partially increased under severe drought but was supported by smoke solution applications, resulting in the highest CP in the 10% solution applications of both drought environments. The increase in CP under severe drought conditions, supported by smoke solution applications, is in line with the findings of (Reinhardt et al., 2015; Yousfi et al., 2016), who also observed an increase in CP under drought stress with smoke treatment. This suggests a potential role of smoke solutions in enhancing protein content under stress conditions. The positive effect of smoke solutions on CP under normal irrigation conditions is consistent with the results of (Dogrusoz, 2022), which showed a positive impact of smoke solutions on protein content even under non-stress conditions.

The ADF ratio increased under stress conditions but was reduced with smoke solution applications. The NDF ratio exhibited different responses under drought intensities, but smoke solutions reduced NDF similarly. These results are in agreement with the findings of (Liu et al., 2018), where smoke solutions were found to reduce fiber content in plants. When Ca, Mg, P, and K minerals were evaluated together, maximum values were observed in the 10% smoke solution application, indicating that smoke solution improved mineral content, especially under severe drought. However, the variable mineral content responses of the population to stress conditions and smoke solutions are consistent with the findings of Pei et al. (2010) and Chen et al. (2011), which also reported variable responses in mineral content under different environmental conditions. These indicate a potential for smoke solutions to improve digestibility and nutritional quality under stress conditions.

Separate PCA biplots for the population and variety of grass pea explained 83.8% and 81.6% of the cumulative variance, respectively. The biplots indicated that smoke solutions diminished the clear differentiation between various treatments, causing them to cluster together. The response mechanism to drought stress in the genotypes was associated with an increase in protein, potassium, and phosphorus. The efficacy of the poppy smoke solution in enhancing drought stress tolerance was highlighted. However, the effect of drought and the defense mechanism of smoke solution created differences in grass pea genotypes. In general, the population was more resistant to drought and the response to smoke solution was stronger.

Plant-derived smoke solutions, renowned for their significant impact on germination, root growth, and seedling development (Dogrusoz, 2022), have been found to effectively alleviate the response of forage peas to drought stress (Li et al., 2017). Recent studies have attributed this protective effect against drought to strigolactones and karrikins butenolide molecules found in plant-derived smoke solutions. These components have been demonstrated to positively influence various processes related to plant drought responses, including the regulation of chemical composition (Li et al., 2017; Yang et al., 2020; Zheng et al., 2020). The application of smoke solutions in the context of drought may assist plants in overcoming stress and enhancing their resilience. However, the specific effects can vary depending on the plant species, doses, and environmental conditions.

In conclusion, this study provides into the positive effects of drought stress and smoke solutions on the biochemical responses and mineral content of grass pea genotypes. However, this effect of the smoke solution was more pronounced on the population and 10% dose is recommended for both stress levels. Thus, this study has proven that the smoke solution has a preventive effect on drought stress, where the future threat is at an alarming level. However, further studies are warranted to elucidate the underlying mechanisms and optimize the use of smoke solutions in sustainable agriculture.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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